Technical Report No. 12

ECOGEOGRAPHICAL VARIATIONS OF CHROMOSOMAL POLYMORPHISM IN HAWAIIAN POPULATIONS OF *DROSOPHILA IMMIGRANS*

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U. S. International Biological Program

February 1973

ABSTRACT

Eighteen samples from twelve populations of Drosophila immigrans in the islands of Kauai, Oahu and Hawaii in the Hawaiian archipelago were analyzed for inversion polymorphism in 1125 females and 206 males. Three kinds of second chromosome inversions, which appear to be identical with those previously reported by other workers, were present in all of our populations; two other new inversions of the same autosome were detected from the Hawaii collections, but their origin, whether natural or laboratory, could not be assured.

The average proportion of inversion heterozygosity per individual of the populations from Kauai, Oahu and Hawaii was about 34%, 32% and 65% respectively. The frequencies of heterozygous inversions were similar between different populations within islands (with one notable exception on Hawaii). In contrast, the frequencies were significantly heterogeneous from one island to the next. The results of gene arrangement frequency analysis consolidated the above findings. It is suggested that the inter-island differentiations are due to natural selection and probably maintained by the isolation by oceanic channels. Two near-by localities on Hawaii were inhabited with significantly heterogeneous populations. Such a microgeographic differentiation has been interpreted as being due to the presence of highly localized, differential selection forces in the two localities, and the difference seems to be maintained due to isolation by the lava flows.

Our data suggest that the breeding units of Hawaiian populations of D. immigrans are not so small as to allow for genetic drift to significantly affect the populations. Inversion polymorphism was similar between females and males taken at the same time in the same localities.

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INTRODUCTION

Over the thirty years since the pioneer work of Dobzhansky, chromosomal inversion polymorphisms have been extensively studied by many workers in various species of Drosophila. Much of the older works are reviewed by Patterson and Stone (1952) and Da Cunha (1955); recent works by Dobzhansky (1961), Carson (1965) and Brncic (1970). Notable among other more recent additions are those on European populations of D. subobscura (Prevosti 1964; Krimbas 1964; Sperlich and Feuerbach 1966); on North American populations of the melanica group (Stalker 1965); and on endemic species of Hawaiian Drosophila (Carson, Hardy, Spieth and Stone 1970).

The geographically localized "wild" species of Drosophila represent over 95% of existing species of the genus (Stone et al. 1960), and many of these are chromosomally highly polymorphic. It has been suggested that the variabilities are utilized as a progressive factor of adaptation to complex environments, assuming quantitative and/or qualitative local changes with vicissitudes (viz., chromosomal polymorphism of the "flexible" type). Some of the wild species contradict this pattern and show either flexibility or rigidity.

On the other hand, most of the "domestic" species which are virtually cosmopolitan in distribution are chromosomally polymorphic at low frequencies, but these variants are less subject to geographic variation (viz., polymorphism of the "rigid" type). Among all the widespread species, only D. funebris in Russia has been known to show polymorphism of the "flexible" type conforming to that of polymorphic endemic species (Dubinin and Tiniakow 1946), whereas in others (e.g., D. simulans, D. virilis and D. repleta) polymorphism is absent. On the whole, the utilization of such adaptational variation tends to be extremely restricted in this widespread species. Thus it appears that the widespread species are restricted in the manner in which they utilize chromosomal polymorphism in adaptation. Questions which logically arise are: 1) what causes some populations

or species to be more polymorphic than others? 2) why is the polymorphism "flexible" in some species but "rigid" in others? and 3) is the "rigid" polymorphism not adaptively important? At present, answers to these questions are far from clear and highly controversial.

Carson (1965) and Dobzhansky (1965) have cited in several instances that some of the colonizing and domestic species have succeeded in establishing themselves in natural habitats, and some wild species have manifested a tendency toward becoming a colonizing species. Among such species, D. immigrans, particularly in the Hawaiian Islands, is of special interest in seeking an answer to the question of the causal connection between adaptation and the characteristics of chromosomal polymorphism. In the Hawaiian Islands, this species is adapted not only for breeding in introduced plants, but also for breeding in unpolluted endemic niches and compete, at least in part, there with the native Hawaiian Drosophila (Heed 1968). Our recent collection data (Paik and Sung 1972a) further showed that the vertical distribution of D. immigrans extends up to 6000 or more feet elevation where it is the predominant Drosophila species. These features have led to the selection of this species for the present study.

In this paper, we intend to analyze data on inversion polymorphism and interpret the observed successful adaptation of D. immigrans to diverse natural habitats in the Hawaiian Islands. Pooling the results of our interlocking studies, and comparing the present data with others so far available, we will seek documentation of the chromosomal basis of adaptation of this species.

A preliminary report of part of this study was presented by the authors in DIS-48 (Paik and Sung 1972b).

MATERIALS AND METHODS

The population samples studied were taken by yeasted banana baits from the

islands of Oahu, Hawaii and Kauai (FIG. 1).

The Oahu samples were from three distinct habitats: the first population (OT-70n) was sampled in late November, 1970, in Mt. Tantalus at 1500-1700 foot levels. A second collection (OT-71j) was undertaken at the same test site in mid-January, 1971. Characteristic trees of this area are introduced ones like guava (Psidium) and avocado (Persea), and extreme humidity characterizes this mountain. Another sample (OM-70o) was collected at Mauna Kapu at about 2300 foot level in late October, 1970; a second collection (OM-71j) and a third collection (OM-71m) in early January and March, 1971 respectively. This mountain is conspicuously dry. Eucalyptus spp. and Schinus terebinthifolius are most common tree species, and guava trees are found scattered in a restricted area. A final sample (OP-71m) was collected in mid-March, 1971 at Puu Keaau at 500-1000 foot levels. Cactus (Opuntia) forms characteristic vegetation of this mountain, with some woody plant species in relatively small numbers.

The Hawaii populations were sampled in early April and late December, 1971, in the vicinity of Hawaii Volcanoes National Park and Kilauea Forest Reserve. These samples will be referred to as HS- and HK- respectively. In the first collecting area samples were taken at six elevations along the Mauna Loa Strip Road extending from 3000 to 6700 feet; in Kilauea Forest at an altitude of approximately 5300 feet. Biotic characteristics of Mauna Loa Strip Road by elevations are: Koa, savanna ecosystem in a matrix of grass communities formed by Paspalum dilatatum as a major component from 4000-4300 feet; from 5110-6100 feet, mountain parkland ecosystem formed by Acacia koa tree colonies, Styphelia-Dodonaea tall scrub communities, both in a matrix of subalpine grassland; and from 6700-7500 feet, open subalpine Metrosideros-Sophora scrub forest. In Kilauea Forest, Acacia koa and Metrosideros collina are the most common tree species, and tree fern (Cibotium spp.) are found scattered throughout the forest. Detailed

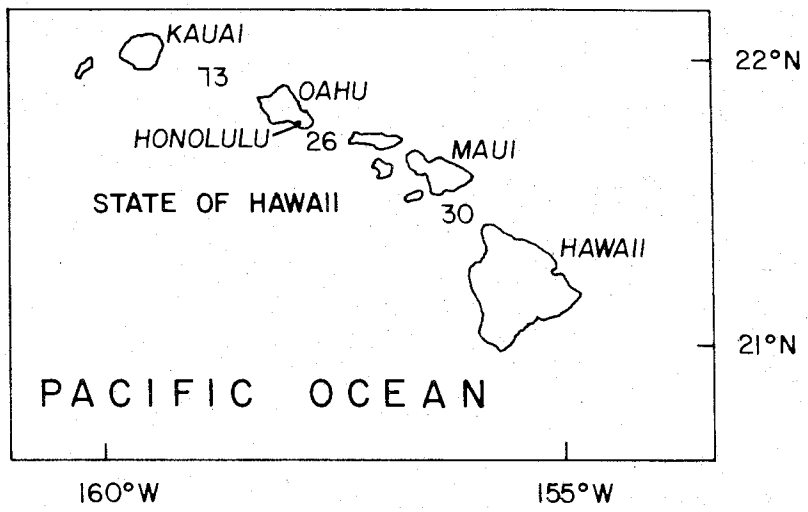

FIG. 1. Map of major islands of Hawaiian archipelago. Arabic figures show the channel distances in miles.

descriptions of the biotic and climatic characteristics of the two regions can be found in Doty and Mueller-Dombois (1966).

The Kauai samples were captured in Mt. Kokee at two different locations on January 23-28, 1972. One collection was made at Halemanu at about 3800 foot level and the other at Berry Flat Trail at approximately 4100 foot elevation. In Halemanu, Acacia koa is the most common tree species; Metrosideros polymorpha is the predominant tree species in Berry Flat Trail, and blackberries are found scattered throughout both areas. The first and second samples will be referred to as KH-72j and KT-72j respectively.

For cytological study, the flies were brought to the laboratory immediately after collection and each female was placed in a separate culture vial. If the wild females had been inseminated, salivary gland chromosomes of one F_1 larva were smeared with the aceto-lacto-orcein from each of the female lines and the slides were then scored for the presence of both heterozygous and homozygous inversions in the fertilized eggs (viz., "egg samples"). The uninseminated wild females were crossed individually to a wild male taken on the same day, and smears of single F_1 larva were made from such cross. The chromosomal constitution of wild males was analyzed sometimes by mating them singly to virgin laboratory females homozygous for "standard" gene arrangement. In this case, salivary gland chromosomes of at least seven F_1 larvae were smeared from each cross (viz., "male adult samples"). From examination of these smears, the distribution of gene arrangements in the wild males was determined. Particular attention was paid to adequate nutrition of larvae by adding a rich yeast solution to the culture medium from time to time. Culture medium consisted of a standard corn meal-molasses-agar mixture plus a small amount of wheat germ containing 0.5% propionic acid as a mold inhibitor. The cultures were kept at $23 \pm 1^{\circ}C$ throughout the experiment.

RESULTS

A total of 1125 females and 206 males of D. immigrans were analyzed for inversion polymorphism from natural populations on the islands of Oahu, Hawaii and Kauai. The salivary gland nuclei of these island populations showed four long euchromatic arms and one euchromatic dot, and one of the long strands was characteristic of its "double-length". No inversions have been found in the X, third or fourth chromosomes.

1. Heterozygous inversion polymorphism

In TABLE 1 the data are summarized on the second chromosome polymorphism in heterozygous condition obtained by "egg sample" technique from the three major islands of Hawaiian archipelago.

We found three kinds of inversions throughout the islands: one is a long median inversion of the left arm of the second chromosome and denoted "A" in TABLE 1 and the subsequent tables. Inversion "B" is a small subterminal one of the right arm, and that designated as "C" is a small proximal inversion of the right arm close to the chromocenter. On Oahu, inversion A is by far the most frequent in occurrence, and the frequency of inversion C is extremely low in each of the six samples. The percentages of the total A and the total B heterozygous inversions in the two combined OT's, three combined OM's and one OP populations are statistically similar (P=0.28 by chi-square test). The pleural (e.g., OT's) is used in the text of this paper and in the tables for samples which have been combined because of statistical homogeneity. The mean number of heterozygous inversions per larva ranged from 0.24 to 0.39 in Oahu populations, with the mean heterozygosity of 0.32. The heterozygosity of all the OM samples appears to be higher than that of all the OT's, with the deviation being significant at 5% level but still not different at the 1% significance level (t=2.451, d.f.=452).

TABLE 1. Frequency of inversion heterozygotes and mean number of heterozygous inversion per larva, measured by "egg sample" technique.

Sample	N	+/+	Percent heterozygous inversions							Mean per larva ± S.E.
			A	B	C	A+B	A+C	B+C	A+B+C	
OT-70n	83	71.1	25.3	2.4	1.2	-	-	-	-	0.29 ± 0.05
OT-71j	120	76.7	18.3	3.3	0.8	0.8	-	-	-	0.24 ± 0.04
OM-70o	53	69.8	24.5	1.9	-	1.9	1.9	-	-	0.34 ± 0.08
OM-71j	158	65.2	22.8	6.3	1.3	2.5	1.3	0.6	-	0.39 ± 0.04
OM-71m	40	70.0	22.5	5.0	-	2.5	-	-	-	0.32 ± 0.08
OP-71m	54	72.2	16.7	5.6	-	3.7	1.9	-	-	0.33 ± 0.08
All Oahu	508	70.5	21.6	4.3	0.8	1.8	0.8	0.2	-	0.32 ± 0.02
HS-4000'a	67	43.3	17.9	10.4	16.4	6.0	-	6.0	-	0.69 ± 0.08
HS-4000'd	167	47.8	17.4	9.0	15.6	6.0	0.6	3.0	0.6	0.63 ± 0.05
HS-5100'a	101	38.6	32.7	9.9	6.9	5.9	3.0	3.0	-	0.73 ± 0.06
HS-5100'd	90	43.4	30.0	10.0	4.4	7.8	3.3	-	1.1	0.71 ± 0.08
HK-5300'a	10	60.0	10.0	10.0	10.0	-	10.0	-	-	0.50 ± 0.22
HK-5300'd	30	40.0	20.0	10.0	10.0	13.3	6.7	-	-	0.80 ± 0.14
HS-4300'a	12	66.7	25.0	-	8.3	-	-	-	-	0.33 ± 0.14
HS-6100'a	11	54.5	36.4	-	9.1	-	-	-	-	0.45 ± 0.16
HS-6700'a	15	59.9	26.7	-	6.7	6.7	-	-	-	0.47 ± 0.16
HS-3000'a	24	70.8	12.5	-	8.3	4.2	-	4.2	-	0.38 ± 0.13
All Hawaii	527	46.5	23.1	8.5	10.8	6.3	1.9	2.5	0.4	0.65 ± 0.03
KH-72j	53	67.9	5.7	11.3	13.2	-	-	1.9	-	0.34 ± 0.07
KT-72j	37	67.6	13.5	2.7	13.5	2.7	-	-	-	0.35 ± 0.09
All Kauai	90	67.8	8.9	7.8	13.3	1.1	-	1.1	-	0.34 ± 0.05

N and +/+ denote number of larvae examined and percent inversionless individuals respectively.

The polymorphism on Hawaii appears to be quantitatively quite unlike that observed on Oahu (see note on TABLE 1). Average number of the heterozygous inversions per larva is generally much higher, ranging from 0.33 to 0.80 in Hawaii populations, with the mean of 0.65. Agreement of this average heterozygosity per larva is striking, both between the two HS-4000' samples and between the two samples of HS-5100' populations. Ostensively, the average individual heterozygosity in the HK-5300' was appreciably low in the April collection as compared with that of the December collection. Nevertheless, the practical significance of this discrepancy seems unimportant: the deviation arose mainly from the excess of heterozygous inversion A (12 out of 30 larvae studied) in the December collection and the corresponding reduction of the incidence (2 out of 10 larvae studied) in the April sample, but the observed variations between these two small samples can be considered as a result of chance, viz., hypergeometric distribution probability is P (Inversion A $\leq$ 2)=0.081. The heterozygosity observed in the other four populations appears generally lower than those of the aforementioned populations, but with too small a sample size in each of the four to make the difference of practical significance. Concordance of the average heterozygosity is also striking on pooled basis, both between HS-5100' and HK-5300' and between HS-4000' and HK-5100' (t=1.166, d.f.=423), where the greatest number of samples were actually examined. Additionally, it was found that the distribution of the total number of heterozygous inversions of each kind was uniform for the April and December samples in both HS-4000' and HS-5100' populations. The chi square tests of independence were made between HS-4000'a and 'd and between HS-5100'a and 'd, based on the compilation of the total number of the three separate inversions from TABLE 1, and the tests show $0.90 > P > 0.80$ and $0.80 > P > 0.70$ for the distribution of the three inversions in HS-4000' and HS-5100' respectively. On the contrary, the chi square test based on the hypothesis of independence between the frequency of the inversion and the

elevation shows a P value of less than 0.001. This result indicates that the distribution of heterozygous inversions is significantly heterogeneous between the two populations. These two collecting sites are separated by only a few miles, but they were isolated by a lava flow about 2000 years ago (Smathers, quoted in Rockwood 1969) and by another more recent flow. While, the distribution pattern of the heterozygous inversions in HK-5300's appears not exactly alike that of either HS-4000's or HS-5100's, but the differences do not attain the conventional level of significance.

Other findings of interest in the Hawaii populations are the detection of two new inversions, which are clearly unlike any described in the available literature, besides the inversions A, B, and C. Both were found on Chromosome 2, one of which was detected from the April sample of Kilauea Forest and the other from the December sample of the HS-5100' collection. The Kilauea inversion is pericentric, relatively short and symmetric about the centromere. It was found in association with the inversion A, but was detected only once in the examination of twenty smear preparations (2 smears per isofemale line) of the F_1 larvae salivary glands of the 10 wild-caught females. The new HS-5100' inversion was found near the proximal region of the right arm and overlapped inversion C, forming a complex inversion. It was also found only once in the examination of ninety F_1 larvae smears (1 smear per isofemale) of the wild-caught females. However, we cannot rule out the possibility that they may be laboratory induced mutations.

In TABLE 1 are shown the polymorphisms in heterozygous state from the Kauai populations. It shows a striking similarity between the two samples in the amount of average individual heterozygosity, with the mean of 0.34. The observed frequencies of heterozygous inversions A, B and C were 5.7%, 13.2% and 15.1% for KH-72j and 16.2%, 5.4% and 13.5% for KT-72j, respectively. However, the observed difference between the two populations can be accepted to be insignificant in both

inversions A and B, probably as a result of errors of sampling. Fisher-Irwin's hypergeometric distribution test gives the significance probability of 0.052 for A and 0.113 for B. It may be legitimate, therefore, to pool the data for these two small samples. The combined frequency of heterozygous inversion C (14.4%) tends to rank the highest among others, and may be considered to be characteristic of populations on the islands of Kauai.

2. Frequency of different gene arrangements

Percentages of the three inverted gene arrangements in all Oahu and Kauai populations and three adequately sampled populations of Hawaii are shown in TABLE 2. On Oahu occurrence of A was most frequent, and C was distinctively low, probably too low to give an accurate estimate of the true proportions. The frequency differences between samples taken at different times in the same localities are not statistically significant. The deviation found between the combined frequency of A or B of the two OT's and that of the three OM's do not quite attain the 1% level of significance. The interpretation of this deviation, however, still requires much caution. Thus, the three ecologically distinct populations from Oahu are nearly alike in the incidence of each gene arrangement.

The gene arrangement frequencies of the three selected Hawaii populations, where the most adequate number of samples could be studied, are presented in TABLE 2. Of the six samples, HS-4000'd and HK-5300'd were subjected to further analysis of "male adult" samples and of "female adult" samples respectively, in addition to the commonly utilized "egg samples". The gene arrangement frequencies are shown in the table on a pooled basis of these two groups. Comparison of the wild-caught males and the egg samples of wild-caught females taken at the same time in the same area has shown no difference in inversion frequencies between the two groups, so that this treatment can probably be acceptable (see note on

TABLE 2. Percentage frequency of different gene arrangements, with the tests of significance of the differences within islands.

Sample		N'	Gene arrangement A	B	C
Oahu:	OT-70n	166	12.60	1.20	0.60
	OT-71j	240	11.67	2.08	0.42
	Pooled	406	12.07 ± 1.61	1.72 ± 0.63	0.49 ± 0.32
	OM-70o	106	20.75	1.89	0.94
	OM-71j	316	15.82 *	4.75 *	1.58
	OM-71m	80	15.00	3.75	-
	Pooled	502	16.73 ± 1.64	3.98 ± 0.84	1.20 ± 0.45
	OP-71m	108	12.96 ± 3.22	4.63 ± 2.00	0.92 ± 0.92
Hawaii:	HS-4000'a	134	14.18	11.19	11.19
	HS-4000'd	454#	15.86	9.25	9.03
	Pooled	588	15.48 ± 1.48	9.69 ± 1.18	9.52 ± 1.18
	HS-5100'a	202	24.26 **	9.40	6.44 **
	HS-5100'd	180	21.11	8.89	3.99
	Pooled	382	22.77 ± 2.14	9.16 ± 1.45	5.24 ± 1.10
	HK-5300'a	20	10.00	5.00	10.00
	HK-5300'd	98$	19.39	9.20	8.20
	Pooled	118	17.80 ± 3.51	8.47 ± 2.55	8.47 ± 2.55
Kauai:	KH-72j	176#	7.39 ± 1.95	6.82 ± 1.90	9.66 ± 2.21
	KT-72j	90#	6.67 ± 2.63	4.44 ± 2.14	7.78 ± 2.81

N' indicates the number of chromosomes tested, in all tables.
Figures include chromosomes from "male adult samples".
$ Figure includes chromosomes from "female adult samples".
*and ** are significant at 5% and 1% level respectively.

TABLE 3). The gene arrangement A is most abundant among the inverted gene sequences in all samples. Inversion C, which was extremely rare in Oahu populations, occurred at much higher frequency in the Hawaii populations; inversions B and C were similar in abundance. The frequency differences in the within-location tests showed no statistical significance for any one of the three arrangements. However, the data given in TABLE 2 show significant differences between HS-4000' and HS-5100' collections in the occurrence of inversions A and C, on pooled basis of the two samples of each location. The frequency of A was higher by 7 to 8% in the HS-5100's than that of the HS-4000's, but conversely the incidence of C was lower by 4 to 5% in the former than that of the latter. The variations, such as these observed, may be interpreted as selective responses to the different environments, as proposed earlier. It does not rule out the possibility, however, that at least part of the variations may also be due to random drift and to the operation of the founder principle.

The gene arrangement frequencies of the two Kauai populations are given in TABLES 2 and 3. The occurrence of gene arrangements are not uniform between the "male adult" and "egg" samples in both populations, but none of the differences is significant at 1 to 5% level. It is hence most likely that the differences between the two types of samples arose through errors of sampling due to their small sample size, and not from a real difference which might exist between the wild-caught males and females. In TABLE 3, the frequencies of arrangement C are similar between the two "male adult samples" from KH-72j and KT-72j; the Fisher-Irwin test for the difference between the two "male adult samples" gives the significance probability of 0.08 and 0.24 for gene arrangements A and B, respectively. Based on the combined data of "egg" and "male adult" samples, the two Kauai populations do not significantly differ at the 5% level in the amount of any of the three gene arrangements (TABLE 2). The frequency of C ranks first both on

TABLE 3. Comparison of gene arrangement frequencies (per cent) between "egg samples" and "male adult samples" in three populations, with the Fisher-Irwin tests of significance of the differences.

Population	N'		Gene arrangement A	B	C
HS-4000'd:					
Male sample	120		17.50	7.50	5.83
Egg sample	334		15.27	9.88	10.18
		P ≃	0.29	0.22	0.08
KH-72j:					
Male sample	70		11.43	7.14	12.86
Egg sample	106		4.72	6.60	7.55
		P ≃	0.05	0.44	0.12
KT-72j:					
Male sample	16		0.00	12.50	12.50
Egg sample	74		8.10	2.70	6.75
		P ≃	0.14	0.04	0.22

a location basis or a pooled basis of the two populations, and the result is conformable to the incidence of heterozygous inversion C which was previously indicated.

3. Test for Hardy-Weinberg distribution

Finally, the data presented in TABLES 1 and 2 are rearranged for each island, combining the same gene sequences, in order to determine if the arrangements observed fit the expectancies on the Hardy-Weinberg rule, on the assumption of random mating and linkage equilibrium. In the pooled sample of all the Oahu populations, the observed numbers of homozygotes and heterozygotes of the various combinations of the separate gene sequences fitted the calculated expectancies.

For Hawaii collections, the combined samples of each of HS-4000', HS-5100' and HK-5300' were tested separately for Hardy-Weinberg distribution. HS-4000' and HK-5300' were in good agreement with the expectation for all the separate arrangements, but HS-5100' showed excess of heterozygous individuals for inversion A at the 1 to 5% significance level (see TABLE 4).

In Kauai collections, the pooled sample of the two populations (KH-72j and KT-72j) conformed to the expectations for all the separate arrangements.

In brief, all the foregoing data indicate that the inversion polymorphism is differentiated quantitatively from one island to the next; while on the same islands the differentiation is generally not clear among ecologically distinct populations, with the one critical exception of the situation found between the two Hawaii populations (HS-4000' and HS-5100'), which will be discussed more in detail later. In TABLE 5 are summarized the inter-island heterogeneities in frequencies of the three separate gene sequences.

DISCUSSION

Two kinds of karyotypes have been recorded for D. immigrans, and possible

TABLE 4. Chi-square test for the significant difference between observed and expected distributions of zygotic frequencies in one Hawaii population.

Population	Genotypes								
	+/+	A/+	A/A	+/+	B/+	B/B	+/+	C/+	C/C
HS-5100'									
Observed	105	80	6	155	36	0	170	21	0
Expected#	113.9	67.2	9.9	157.6	31.8	1.6	171.5	18.9	0.5
Difference	-8.9	12.8	-3.9	-2.6	4.2	-1.6	-1.5	2.1	-0.5
$P_X2 \simeq$		0.03*			0.50			0.73	

On the assumption of linkage equilibrium of different gene arrangements.

TABLE 5. Inter-island heterogeneity in frequencies of gene arrangements, with the tests of significance.

Comparison	Ins. A		Ins. B		Ins. C	
	R*	X^2	R	X^2	R	X^2
Oahu's vs. Kauai's	14.47/7.14	10.07***	3.15/6.02	4.89*	0.88/9.02	55.77***
Oahu's vs. HS-4000's	14.47/15.48	0.30	3.15/9.69	30.37***	0.88/9.52	71.50***
Oahu's vs. HS-5100's	14.47/22.77	13.70***	3.15/9.16	22.03***	0.88/5.24	25.99***
Oahu's vs. HK-5300's	14.47/17.80	1.02	3.15/8.47	8.45**	0.88/8.47	36.75***
Kauai's vs. HS-4000's	7.14/15.48	11.37***	6.02/9.69	3.13	9.02/9.52	0.001
Kauai's vs. HS-5100's	7.14/22.77	27.95***	6.02/9.16	2.14	9.02/5.24	3.56
Kauai's vs. HK-5300's	7.14/17.80	9.93**	6.02/8.47	0.79	9.02/8.47	0.04

R* shows the ratio of percentage frequency between the indicated populations. For the estimates of gene arrangement frequencies, Kauai's and HS-4000's included "male adult samples" and HK-5300's included "female adult samples." *, **, *** are significant at 5%, 1% and 0.1% level respectively.

cytological explanations for the occurrence of different types have been advanced by Patterson and Stone (1952). Type 1 has been found by many different workers from different countries; their salivary gland nuclei show four long arms and one dot, and one of the long strands is characterized by its double length as compared with normal length of the other three long arms. Type 2 was reported by only two workers (Emmens 1937; Wharton 1943), with the salivary chromosome configurations showing five long arms and one dot. The salivary gland chromosomes in our populations from Hawaii, Oahu and Kauai, as far as they have been tested, were found to be identical with those of type 1. Richmond and Dobzhansky (1968) also reported type 1 configuration for the populations from Maui, another island of the Hawaiian archipelago.

At least three different inversions of Chromosome 2 are known to be "universal" in their occurrence. These same inversions occurred in all the populations from Hawaii, Oahu and Kauai, as shown in the RESULTS, where they are denoted as inversions A, B and C. Richmond and Dobzhansky (1968) also found the same result for the populations on Maui. Our findings of the new endemic inversions in the Kilauea Forest population and the Mauna Loa Strip Road population at 5100' elevation are of some interest for further study.

The mean frequencies of inversion heterozygosity per larva in the total Oahu population (0.32) and the whole Kauai population (0.34) are quite similar to each other, but the corresponding figure for the Hawaii population (0.65) is about twice as high as those found in the two other islands. The differences between Hawaii and Oahu and between Hawaii and Kauai are in both cases significant (t-test shows P values of less than 0.001 for both). These data suggest that the Oahu and Kauai populations are at a different level of fitness than the Hawaii populations studied, and they are probably under stronger environmental selection pressure, as will be exemplified a little later.

The inter-island heterogeneity can be accentuated by comparing the distribution of the heterozygous inversions between islands: distribution of the inversions A, B and C is significantly nonuniform between the data for Oahu's and that for HS-4000's on Hawaii (X^2=52.96, d.f.=2, $P < 0.001$), between Oahu's and HS-5100's, Hawaii (X^2=11.82, d.f.=2, $P < 0.001$), between Oahu's and Kauai's (X^2=40.23, d.f.=2, $P < 0.001$), and between Kauai's and HS-5100's (X^2=13.20, d.f.=2, $0.01 > P > 0.001$). Incidentally, Kauai's and HS-4000's show no statistical difference in this respect (X^2=1.52, d.f.=2, $0.50 > P > 0.30$), but they are sharply different from each other in their average individual heterozygosity. Inter-island heterogeneity is also evident in the data on gene sequence frequencies (see TABLE 5).

HS-4000's and HS-5100's have been singled out from the Hawaii populations as crucial for our comparison, since 1) their sample sizes are most adequate for the statistical treatment, 2) the distribution of the heterozygous inversions is significantly different at 1% level between the two populations, and 3) the data of gene sequence frequency reveal striking difference between the populations. The occurrence of such striking heterogeneity between the two populations situated only a few miles apart in the same mountainside is suggestive of differential selection. There was also some indication of heterogeneity on Oahu and on Maui (Richmond and Dobzhansky 1968), though not reaching the strict level of significance.

Variations in the incidence of heterozygous inversions among the widely separated natural populations of D. immigrans, studied by different workers, are summarized in FIG. 2. There are considerable differences not only in the frequency of each inversion but also the mean individual heterozygosity among populations. Freire-Maia et al. (1953) detected only one kind of inversion (A) in eight local populations from central and southern Brazil. Mean number of the heterozygous inversions per larva was 0.20. In the widely separated populations of Chilean

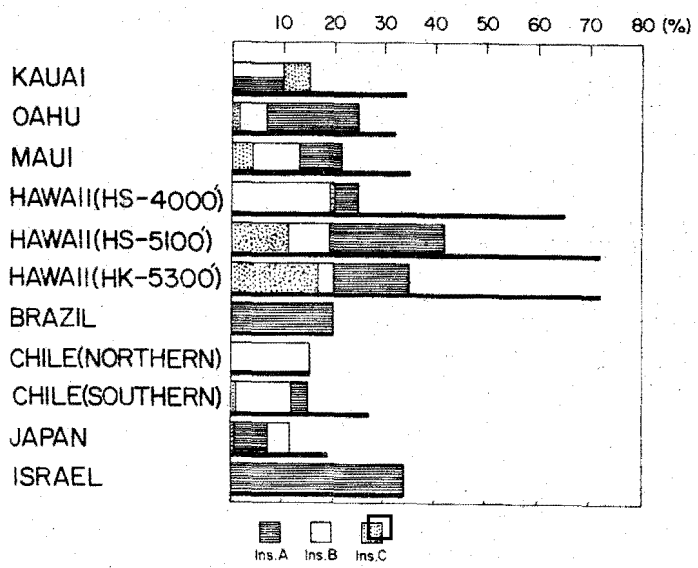

FIG. 2. Comparison of geographic variations in heterozygous inversion frequencies. The horizontal ▬ indicates the average proportion of inversion heterozygosity per individual of the indicated population.

D. immigrans inversions A, B and C were found by Brncic (1955). Mean number of the heterozygous inversions per larva reported by Brncic ranged from 0.10 to 0.43, with the mean of 0.20. On pooled basis, inversion B is most frequent in occurrence and distributes throughout Chile, and its average heterozygous frequency being close to 13%; frequency of heterozygous inversion A is about 7% (including the strains showing no chromosomal polymorphism at all) and restricted to Southern Chile in distribution; inversion C was found in only one of the ten populations, and even then not reaching a substantial frequency. Hirumi (1961) also found the same inversions in Japan with a mean heterozygosity of 0.19 per larva. Similar results were obtained by Toyofuku (1961) in other Japanese populations. Thus Japanese populations resemble Chilean populations in that inversion B is most common and inversion C rarely achieves substantial frequency. Gruber (1958) found Israeli populations strikingly different in that inversion A is the only aberration present (with the mean individual heterozygosity close to 0.34). The Israeli population is qualitatively similar to the Brazil but quantitatively unlike the latter. The polymorphism found in the island of Maui by Richmond and Dobzhansky (1968) are qualitatively but not quantitatively the same as found in Chile and Japan. The mean frequency of individual heterozygosity in the populations of Maui (0.35) is nearly twice as high as those found in Japan or Chile. Additionally, the most frequent chromosome variant on Maui is inversion A (21%) unlike the situations found in Japan and Chile, where inversion B is the most common (12-13%). Further, the Maui populations resemble populations from Oahu and Kauai but are clearly different from the populations on Hawaii in inversion heterozygosity. While, distribution of the three heterozygous inversions are significantly different between Maui and Oahu (X^2=14.43, d.f .=2, $P < 0.001$) and between Maui and Kauai (X^2=20.24, d.f.=2, $P < 0.001$). The difference between Maui and HS-4000's on Hawaii is also striking (X^2=21.20, d.f.=2, $P < 0.001$), even though the populations of Maui and HS-5100's cannot be differentiated by this index at the 1 to 5% level of significance. More interesting still is the

finding that the populations on Kauai are characterized with the most frequent occurrence of inversion C (15%), which is generally known to be most rare or hardly achieves substantial frequency in other localities.

From this brief review, it may be safe to conclude that inversion polymorphisms of D. immigrans are by no means uniform in response to local or geographical environmental conditions, and the differences between populations are quantitative far more often than qualitative. However, the interpretation of the local variations still requires due consideration of the effect of random-drift or founder principle.

Spencer (1947), from his study on the frequency and distribution of visible mutations in North American populations of D. immigrans, concluded that they are chiefly small, discrete breeding units, and in which genetic drift is pronounced due to periodic fluctuations in population size related to the winter season. However, our tests of Hardy-Weinberg distribution of the polymorphism suggest that the breeding units of Hawaiian populations of this species are not so small as to allow genetic drift to significantly affect the inversion frequencies. HS-5100' on Hawaii is of some interest since it is the only population which exhibited apparent deviation from the Hardy-Weinberg expectancy among our quantitative samples. The difference is due mainly to the excess of individuals which are heterozygous for inversion A. This finding appears to fulfill Carson's idea (1959, 1965) stressing the importance of heteroselection in the maintenance of "rigid" polymorphism.

Dobzhansky et al. (1950) first showed that the amount of inversion polymorphism in South American populations of D. willistoni is positively correlated to the richness of natural resources which the species can utilize. Brncic's (1955) data suggest a similar situation in the cosmopolitan species, D. immigrans, in Chile. He found the mean frequency of individual inversion heterozygosity was

about 0.4 in the southern populations inhabiting the rich rainforest but only 0.15 in the populations from northern Chile where the species breeds in the drastic environments of desert. Moreover, the southern populations had inversions A, B and C whereas the only B was present in the northern populations. The sharp contrast between the southern and northern populations appears to be related to the differential heterogeneity of the two environments. The high proportion of inversion heterozygosity per individual and the simultaneous occurrence of all the three types of inversions in Hawaiian populations of D. immigrans may reflect richer conditions of ecological environment on the Hawaiian Islands as compared to the continental areas studied.

It is difficult to single out a factor(s) which is responsible for local or geographical differentiation of chromosomal polymorphisms. However, our finding of the significant heterogeneity between the two spatially close populations (viz., HS-4000' and HS-5100' on Hawaii) may reasonably be interpreted as being the consequence of highly localized, differential selection pressures in the micro-geographic isolation as formed by lava flows. The available eco-climatic data (see Doty and Mueller-Dombois (1966), and unpublished weekly climatic data of the University of Hawaii's IBP station in Mauna Loa Strip Road) suggest that between the two locations sufficient differences exist in vegetation and microclimates (temperature, rainfall and humidity) to promote differential selection. Additionally, the notably high proportion of individual inversion heterozygosity in populations on Hawaii compared to other Hawaiian islands may be related to bio-climatic environmental differences. The Hawaii populations have two properties relevant to this situation. First, the Hawaii populations originated from substantially higher altitudes than those studied on the other islands. Secondly, Hawaii populations may experience less competition with other sympatric species of Drosophila than do populations of D. immigrans on the other islands. On

Kauai, Oahu and Maui, D. immigrans coexisted with and was subordinate to other introduced species such as D. nasuta, D. simulans, D. sulfurigaster, D. busckii, D. hydei and/or D. mercatorum. In contrast, in the Hawaii collections D. immigrans was predominant and made up at least 70 to 80% of the total flies captured (Paik and Sung 1972a). D. simulans was second in abundance (10-20%); other introduced species were absent except for the unexpected occurrence of D. busckii in one of the two collections at the HS-5100' study site. Many Hawaiian endemic Drosophilids were also attracted to the baits, but never in large numbers. Heed (1968) and Montgomery (unpublished data, 1972) from their study of breeding sites of Hawaiian Drosophila have shown that D. immigrans utilize fruits, and occasionally stems, leaves and barks, of at least 9 species of endemic plants and many other exotic plants as breeding sites. Their records, however, indicate that many of these materials are of only minor importance as breeding sites of majority of endemic species of Hawaiian Drosophila. Da Cunha et al. (1959) from their study of inversion frequency in D. willistoni and Ives (1954) from the similar work in D. melanogaster have shown that the presence of related species impedes genetic variations. Competition of related species has recently been stressed by Mayr (1970) as a particularly important factor for movement into new habitats or ecological niches. In the light of these information, the D. immigrans data suggest that much of the reduced individual heterozygosity in populations on Kauai, Oahu and Maui, compared to the Hawaii, is related at least in part to the selection pressure due to the presence of competitors. Available information such as these, together with the observable magnitude of invasion (or abundance) of D. immigrans in the endemic habitats on Hawaii, suggests that this species has largely exploited "empty" niches on Hawaii without facing any real resistance of the endemic flies. Such interpretation, in turn, supports our earlier view that has proposed an inverse relationship between competition and inversion heterozygosity.

In conclusion, although D. immigrans is a cosmopolitan species, it does not appear to be quite as specialized ecologically as other cosmopolitan species. Although the number of chromosomal inversion types is restricted in this species, the polymorphism appears to respond to different local ecological and bio-climatic conditions. Further analysis of the genetic basis, on both genic and chromosomal level, of this adaptation to diverse habitats is presently in progress.

ACKNOWLEDGMENTS

We are most grateful to Professor H. L. Carson for calling this research problem to our attention and for his valuable comments and criticisms on the manuscript. Sincere appreciation is extended to Professor Th. Dobzhansky of the University of California for his invaluable suggestions and criticisms of the manuscript, to Dr. W. E. Johnson for his helpful discussions in preparing the manuscript, to Professor G. E. Ashton for his support and encouragement, and to Mr. K. Kaneshiro of Department of Entomology for the identification of Drosophila species. We are also grateful to Mr. S. L. Montgomery of Department of Entomology for providing his unpublished data.

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